

Amendments to the Claims:

This Listing of Claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

Claims 1-49 (canceled).

Claim 50(currently amended). A plasmid DNA polynucleotide which is non-replicating[[,]] upon *in vivo* introduction into a mammalian cell, ~~is non-replicating~~ and induces the co-expression in the cell of at least two and not more than three gene products, comprising:

- a) a first transcriptional promoter which operates in eukaryotic cells upstream from, and in transcriptional control of, a first cistron;
- b) a second cistron downstream from the first cistron, under transcriptional control either of the first transcriptional promoter or under control of a second transcriptional promoter;
- c) optionally, a third cistron downstream from the second cistron, under transcriptional control either of the first transcriptional promoter or under control of the second transcriptional promoter, or under control of a third transcriptional promoter; and,
- d) a transcriptional terminator following each of the first, second and third cistron, unless said first cistron or second cistron is followed by a second cistron or third cistron, respectively, which lacks its own transcriptional promoter and wherein at least either the first, second or third cistron encodes at least one immunogenic epitope of a human immunodeficiency virus antigen.

Claim 51(currently amended). The plasmid DNA polynucleotide of Claim 50 wherein the first cistron encodes a human immunodeficiency virus (HIV) gene selected from the group consisting of env, gag, gag/pol, gag/protease, gag and portions of pol not encoding a functional polymerase, and pol.

Claim 52(currently amended). The plasmid DNA polynucleotide of Claim 50 wherein the second cistron encodes a human immunodeficiency virus (HIV) REV gene if the first cistron encodes an HIV gene, the efficient expression of which is dependent on availability within the cell expressing the HIV gene of the REV gene product.

Claim 53(currently amended). The plasmid DNA polynucleotide of Claim 52 wherein the first cistron encodes an HIV late gene selected from env, gag and pol.

Claim 54(currently amended). The plasmid DNA polynucleotide of Claim 53 wherein the first cistron encodes an HIV antigen selected from the group consisting of HIV gp160, HIV gp120, HIV gp41, HIV gp120 lacking a CD4 binding site and ,an HIV env with an immunologically altered V3 glycosylation pattern, and an HIV env with the altered V3 ~~having an altered glycosylation pattern or~~ substituted V3 loop tips.

Claim 55(currently amended). The plasmid DNA polynucleotide of Claim 52 wherein the third cistron encodes a cytokine or a T-cell costimulatory element.

Claim 56(currently amended). The plasmid DNA polynucleotide of Claim 55 wherein the cytokine is interferon, GM-CSF, or interleukin.

Claim 57(currently amended). The plasmid DNA polynucleotide of Claim 55 wherein the T-cell costimulatory element is a gene encoding a B7 protein.

Claim 58(currently amended). The plasmid DNA polynucleotide of Claim 50 wherein the first cistron encodes a REV-independent human immunodeficiency (HIV) epitope, the second cistron encodes a cytokine, and the third cistron encodes a T-cell costimulatory element, wherein the first, second and third cistron may be presented in any combination.

Claim 59(currently amended). The plasmid DNA polynucleotide of Claim 58 wherein the second cistron encodes an interleukin, an interferon, or GM-CSF, and the third cistron encodes a B7 protein.

Claim 60(currently amended). The plasmid DNA polynucleotide of Claim 50 wherein either of the second and third cistron is under transcriptional control of the transcriptional promoter upstream of the first cistron, a sequence is provided upstream of each of the second and third cistrons having the function of an internal ribosome entry site (IRES) to effect efficient translation of the second and third cistrons on a bi- or tri-cistronic messenger RNA transcribed from the beginning of the first cistron through each of the

second and third cistrons up to the transcriptional terminator following the second or third cistron.

Claim 61(currently amended). The plasmid DNA polynucleotide of Claim 60 wherein the IRES is selected from encephalomyocarditis virus (EMCV) IRES, swine vesicular virus IRES and poliovirus IRES.

Claim 62(currently amended). The plasmid DNA polynucleotide of Claim 60 wherein the first cistron encodes a human immunodeficiency virus (HIV) REV dependent gene, the second cistron encodes REV, and the third cistron encodes a T-cell costimulatory element or a cytokine, and further, wherein the first cistron is preceded by a transcriptional promoter and the second and third cistrons are each preceded by an IRES and no transcriptional promoter.

Claim 63(currently amended). The plasmid DNA polynucleotide of Claim 62 wherein the first cistron encodes an HIV gp160, the first cistron is preceded by cytomegalovirus immediate early promoter, the second cistron encodes HIV REV, the optional third cistron encodes an interferon, GM-CSF, an interleukin, or a B7 protein.

Claim 64(currently amended). A plasmid DNA polynucleotide which cannot replicate in eukaryotic cells *in vivo* and which comprises contiguous nucleic acid sequences capable of being expressed to produce a gene product upon introduction of the polynucleotide into eukaryotic tissues *in vivo*, wherein the gene product either acts as an immunostimulant or as an antigen capable of generating an immune response, wherein the nucleic acid sequences encode:

- a) a spliced REV gene;
- b) a spliced human immunodeficiency virus (HIV) immunogenic epitope; and,
- c) optionally, a cytokine or a T-cell recognition element.

Claim 65(currently amended). The plasmid DNA polynucleotide of Claim 64 wherein the HIV immunogenic epitope of step b) is a gene product expressed from an HIV gene selected from the group of HIV genes consisting of gag, gag-protease, and env or an immunogenic subportion thereof; the cytokine is interleukin-12, and the T-cell costimulatory element is a B7 protein.

Claim 66(currently amended). The plasmid DNA polynucleotide of Claim 65 wherein the env immunogenic epitope is a gene product expressed from an env open reading frame selected from the group consisting of HIV gp160, HIV gp120 and HIV gp41.

Claim 67(currently amended). The plasmid DNA polynucleotide of Claim 65 wherein the gag immunogenic epitope is p17, p24, or p15.

Claim 68(currently amended). A plasmid DNA polynucleotide comprising a first gene encoding an HIV gag, gag-protease, or env immunogenic epitope, the first gene containing a REV responsive element (RRE) or having been modified to contain an RRE, the first gene being operatively linked with a transcriptional promoter suitable for gene expression in a mammal, the first gene being linked with an internal ribosome entry site (IRES), and the IRES being linked with a second gene encoding a REV gene product, wherein said polynucleotide is non-replicating in eukaryotic cells *in vivo*.

Claim 69(canceled).

Claim 70(currently amended). A plasmid DNA polynucleotide which is non-replicating in eukaryotic cells *in vivo*, comprising:

- a) a eukaryotic transcriptional promoter;
- b) an open reading frame 3' to the transcriptional promoter encoding an immunogenic HIV epitope wherein the open reading frame has a splice donor sequence at the 5'-side of the open reading frame, a REV responsive element anywhere within the open reading frame, and a stop codon encoding the termination of translation of the open reading frame;
- c) an internal ribosome entry site (IRES) 3' to the translation stop codon of the open reading frame;
- d) an open reading frame encoding a spliced HIV REV gene at the 3' end of which is a translation stop codon;
- e) optionally, 3' to the REV translation stop codon, a second IRES, followed by an open reading frame encoding immunomodulatory or immunostimulatory genes being selected from the group consisting of GM-CSF, IL-12, interferon, and a B7 protein; and,
- f) a transcription-termination signal 3' of the most downstream open reading frame of step d) or optionally, step e).

Claim 71(currently amended). A plasmid DNA polynucleotide which is non-replicating in eukaryotic cells *in vivo*, comprising sequences encoding:

- a) a eukaryotic transcription initiation signal;
- b) an HIV gene open reading frame (ORF) preceded by a heterologous leader sequence such that expression of the HIV gene ORF does not depend on availability of the HIV REV gene product;
- c) a sequence which operates as an internal ribosome entry site (IRES) 3' to the translation stop codon of the HIV ORF;
- d) a sequence encoding an ORF of a T-cell costimulatory element 3' to the IRES; and
- e) a transcription termination signal 3' to the translation stop codon of the T-cell costimulatory element.

Claim 72(currently amended). The plasmid DNA polynucleotide of Claim 71 wherein the HIV gene ORF in (b) is tPAgp120 or tPAgp160.

Claim 73(currently amended). A plasmid DNA polynucleotide which is non-replicating in eukaryotic cells *in vivo*, comprising sequences encoding:

- a) a eukaryotic transcription initiation signal;
- b) a first HIV gene open reading frame (ORF) preceded by a heterologous leader sequence such that expression of the HIV gene ORF does not depend on availability of the HIV REV gene product;
- c) a sequence which operates as an internal ribosome entry site (IRES) 3' to the translation stop codon of the first HIV ORF;
- d) a second HIV gene open reading frame (ORF) preceded by a heterologous leader sequence such that expression of the second HIV gene ORF does not depend on availability of the HIV REV gene product; and
- e) a transcription termination signal 3' to the translation stop codon of the second HIV gene ORF.

Claim 74(currently amended). A plasmid DNA polynucleotide which is non-replicating upon *in vivo* introduction into a mammalian cell, ~~is non-replicating~~ and induces the co-expression in the cell of at least two and not more than three gene products, the polynucleotide comprising a first transcriptional promoter which operates in eukaryotic cells upstream from, and in transcriptional control of, a first cistron, a second cistron downstream from the first cistron, under transcriptional control either of the first transcriptional promoter or under control of a second transcriptional promoter, optionally, a third cistron downstream from the second cistron, under transcriptional control either of the first transcriptional promoter or under control of the second transcriptional promoter, or under control of a third transcriptional promoter, and a transcriptional terminator following each of the first, second and third cistron, unless said-first cistron or second cistron is followed by a second cistron or third cistron, respectively, which lacks its own transcriptional promoter; wherein each of the first, second and optionally third cistrons encode a combination of any two to three of the following:

- 1) tPA-gp120_{MN};
- 2) gp160_{IIIB}/IRES/*REV*_{IIIB};
- 3) gp160_{IIIB};
- 4) *REV*_{IIIB}
- 5) *tat/REV/gp160*;
- 6) *REV/gp160*;
- 7) gp160_{MN};
- 8) gp160 from a clinical HIV isolate;
- 9) *nef*, obtained from a clinical HIV isolate;
- 10) *gag*_{IIIB};
- 11) tPA-gp120_{IIIB};
- 12) gp160 with structural mutations selected from the group consisting of V3 loop substitutions from a clinical HIV isolate, Asn mutations to remove steric carbohydrate obstacles to structural, neutralizing antibody epitopes; and CD4 binding site knockout mutants;
- 13) gp41 with a signal peptide leader sequence;
- 14) *gag/REV/gp160*;
- 15) B7;
- 16) GM-CSF;
- 17) an interleukin; and,
- 18) a tumor associated antigen.

Claim 75(currently amended). A plasmid DNA polynucleotide construct selected from the group consisting of V1Jns-(tat/rev SD), V1Jns-gp160_{IIIB}/IRES/rev _{IIIB} (SD), V1Jns-gag-prt_{IIIB} (SD), V1Jns-gag-prt_{IIIB}, V1Jns-tPA, V1Jns-tPA-gp120_{MN}, V1J-SIV_{MAC251}p28 gag, V1J-SIV_{MAC251}nef, and V1Jns-tat/rev/env.

Claim 76(currently amended). The plasmid DNA polynucleotide of Claim 50 wherein the first cistron contains an HIV *gag* gene or portion thereof which encodes a *gag* immunogenic epitope, the second cistron encodes a cytokine, and the third cistron encodes a T-cell costimulatory element, wherein the first, second and third cistron may be presented in any combination.

Claim 77(currently amended). The plasmid DNA polynucleotide of Claim 76 wherein the second cistron encodes an interleukin, an interferon, or GM-CSF, and the third cistron encodes a B7 protein.